CLAIMS

What is claimed is:

1. A method of amplifying a template DNA molecule comprising: incubating said template DNA molecule with a reaction mixture comprising a DNA polymerase and at least one accessory protein at a constant temperature to produce amplified product, wherein production of amplified product does not require exogenously-added oligonucleotide primers and said template DNA molecule does not have a terminal protein covalently bound to either 5' end.

2. The method of claim 1, wherein said DNA polymerase is a bacteriophage DNA polymerase.

3. The method of claim 1, wherein said DNA polymerase is bacteriophage T7 DNA polymerase.

4. The method of claim 3, wherein said DNA polymerase is a mixture of wild-type T7 DNA polymerase and a T7 DNA polymerase modified to have reduced 3′ to 5′ exonuclease activity.

5. The method of claim 1, wherein said accessory protein is a helicase.

6. The method of claim 1, wherein said accessory protein is a primase.

7. The method of claim 1, wherein said accessory protein is the helicase/primase of bacteriophage T7.

8. The method of claim 7, wherein said helicase/primase is the 63-kDa form of the protein from bacteriophage T7.

9. The method of claim 1, wherein said accessory protein is a single-stranded DNA binding protein.

10. The method of claim 9, wherein said single-stranded DNA binding protein is from *Escherichia coli*.

11. A method of amplifying a template DNA molecule comprising:
incubating said template DNA molecule with an *in vitro* reaction mixture comprising a DNA polymerase, a helicase, and a primase at a constant temperature to produce amplified product.

12. The method of claim 11, wherein said reaction mixture does not require exogenously-added oligonucleotide primers.

13. The method of claim 11, wherein said polymerase is from bacteriophage T7.

14. The method of claim 13, wherein said DNA polymerase is a mixture of wild-type T7 DNA polymerase and a T7 DNA polymerase modified to have reduced 3′ to 5′ exonuclease activity.

15. The method of claim 11, wherein said helicase and said primase is the helicase/primase of bacteriophage T7 .

16. The method of claim 15, wherein said helicase/primase is the 63-kDa form of the protein from bacteriophage T7.

17. The method of claim 11, wherein said reaction mixture further comprises a single-stranded DNA binding protein.

18. The method of claim 17, wherein said single-stranded binding protein is from *Escherichia coli.*

19. The method of claim 11, where the amount of DNA produced is a 1,000-fold increase over the amount of the template DNA.

20. The method of claim 11, where the amount of DNA produced is a 10,000-fold increase over the amount of the template DNA.

21. The method of claim 11, where the amount of DNA produced is a 100,000-fold increase over the amount of the template DNA.

22. The method of claim 11, where the amount of DNA produced is a 1,000,000-fold increase over the amount of the template DNA.

23. The method of claim 11, where the amount of DNA produced is a 10,000,000-fold increase over the amount of the template DNA.

24. A method of amplifying a template DNA molecule comprising:

incubating said template DNA molecule in an *in vitro* reaction mixture comprising a wild-type T7 DNA polymerase and a T7 DNA polymerase modified to have reduced 3′ to 5′ exonuclease activity, a 63-kDa form of a gene 4 protein from bacteriophage T7 and a single-stranded binding protein from *Escherichia coli* at a constant temperature to produce amplified product.

25. The method of claim 24, wherein said reaction mixture does not require exogenously-added oligonucleotide primers.

26. The method of claims 1, 11, or 24, wherein said amplification of said template DNA is exponential.

27. The method of claims 1, 11, or 24, wherein said reaction mixture further comprises a single-stranded DNA binding protein of bacteriophage T7.

28. The method of claims 1, 11 or 24, wherein said reaction mixture further comprises a nucleoside diphosphokinase.

29. The method of claim 28, wherein said nucleoside diphosphokinase is from *Escherichia coli*.

30. The method of claims 1, 11 or 24, wherein said reaction mixture further comprises an inorganic pyrophosphatase.

31. The method of claims 1, 11 or 24, wherein said reaction mixture further comprises an ATP regeneration system.

32. The method of claim 31, wherein said ATP regeneration system consists of phosphocreatine and creatine kinase.

33. The method of claims 1, 11 or 24, wherein said reaction mixture further comprises a 5' to 3' exonuclease.

34. The method of claim 33, wherein said exonuclease is T7 gene 6 exonuclease.

35. The method of claims 1, 11 or 24, wherein said reaction mixture further comprises a DNA ligase.

36. The method of claim 35, wherein said ligase is T7 DNA ligase.

37. The method of claims 1, 11 or 24, wherein said template DNA molecule has a length of at least 1,000 bp.

38. The method of claims 1, 11 or 24, wherein said template DNA molecule has a length of at least 1,500 bp.

39. The method of claims 1, 11 or 24, where in said template DNA molecule has a length of at least 2,000 bp.

40. The method of claims 1, 11 or 24, wherein said template DNA molecule has a length of at least 3,000 bp.

41. The method of claims 1, 11 or 24, wherein said template DNA molecule is amplified at least about 10-fold.

42. The method of claims 1, 11 or 24, wherein said template DNA molecule is amplified at least about 100-fold.

43. The method of claims 1, 11 or 24, wherein said template DNA molecule is amplified at least about 1000-fold.

44. The method of claims 1, 11 or 24, wherein said constant temperature is less than 60° C.

45. The method of claims 1, 11 or 24, wherein said constant temperature of less than 45° C.

46. The method of claims 1, 11 or 24, wherein said amplification requires the presence of ATP and CTP.

47. The method of claims 1, 11 or 24, wherein said reaction mixture further comprises 0.5 to 5% DMSO.

48. The method of claims 1, 11 or 24, wherein said reaction mixture contains 0.5-5% dextran.

49. The method of claims 1, 11 or 24, wherein said template DNA molecule is from lysed *E. coli* cells.

50. The method of claim 49, wherein said DNA is a plasmid.

51. The method of claim 49, wherein said DNA is a bacterial artificial chromosome (BAC).

52. The method of claim 1, further comprising the step of treating said polymerase and at least one accessory protein with ultraviolet light prior to incubating with said template DNA molecule.

53. The method of claim 11, further comprising the step of treating said polymerases, said helicase, and said primase with ultraviolet light prior to incubating with said template DNA molecule.

54. The method of claim 24, further comprising the step of treating said polymerases, said gene 4 protein and said single-stranded binding protein with ultraviolet light prior to incubating with said template DNA molecule.

55. The method of claims 52, 53, or 54, wherein said ultraviolet light is a dose from 10 to 1000 $\mu W/cm^2$ for from 15 sec to 5 min.

56. The method of claim 1, 11 or 24, wherein said template DNA is less than 100 ng of a plasmid and said reaction mixture is 10 to 200 μl.

57. A method for preparing a DNA molecule for use in a DNA sequencing reaction comprising the steps of:

incubating said DNA molecule in an *in vitro* reaction mixture comprising a DNA polymerase, a helicase, and a primase at a constant temperature so as to produce amplified product; and

providing said amplified product for use in a DNA sequencing reaction.

58. The method of claim 57, wherein said reaction mixture further comprises a single-stranded binding protein.

59. The method of claim 57, wherein said reaction mixture does not require exogenously-added oligonucleotide primers.

60. The method of claim 57, wherein production of said amplified product is exponential.

61. The method of claim 57, wherein the amount of said amplified product is at least 10-fold greater than said DNA molecule put into said reaction mixture.

62. A method for preparing a DNA molecule for use in a DNA sequencing reaction comprising the steps of:

incubating said DNA molecule in an *in vitro* reaction mixture comprising a T7 polymerase and a T7 gene 4 protein at a constant temperature so as to produce amplified product; and

providing said amplified product for use in a DNA sequencing reaction.

63. The method of claim 62, wherein said polymerase is a mixture of wild-type T7 DNA polymerase and a T7 DNA polymerase modified to have reduced 3′ to 5′ exonuclease activity.

64. The method of claim 62, wherein said T7 gene 4 protein is 63-kDa form of the protein.

65. The method of claim 62, wherein said reaction mixture further comprises a single-stranded binding protein from *Escherichia coli.*

66. The method of claim 62, wherein the amount of said amplified product is amplified is at least 10-fold greater than said DNA molecule put into said reaction mixture.

67. The method of claims 57 or 62, wherein said production of said amplified product is exponential.

68. The method of claim 62, wherein said reaction does not require exogenously–added oligonucleotide primers

69. The method of claims 57 or 62, wherein said the DNA sequencing reaction uses fluorescent primers.

70. The method of claims 57 or 62, wherein said DNA sequencing reaction uses fluorescent dideoxynucleotides.

71. The method of claims 57 or 62, wherein dNTPs present in said reaction mixture are removed after amplification by treatment with phosphatase.

72. The method of claim 71, wherein said phosphatase is from arctic shrimp.

73. The method of claims 57 or 62, wherein said DNA molecule is from lysed *E. coli* cells.

74. The method of claim 73, wherein said DNA is a plasmid.

75. The method of claim 73, wherein said DNA is a bacterial artificial chromosome (BAC).

76. The method of claim 73, wherein said DNA is from a human patient.

77. The method of claims 57 or 62, wherein said reaction mixture further comprises DMSO at a concentration of 0.5 to 8%.

78. The method of claims 57 or 62, wherein said reaction mixture further comprises dextran at a concentration of between 0.5 and 20%.

79. A kit for amplification, comprising:
a DNA polymerase;
a primase;
a helicase; and
a single-stranded binding protein.

80. The kit of claim 79, wherein said polymerase is T7 DNA polymerase.

81. The kit of claim 79, wherein said primase and helicase are a gene 4 protein from bacteriophage T7.

82. The kit of claim 79, wherein said single-stranded binding protein is from *Escherichia coli*.

83. The kit of claim 80, wherein said T7 polymerase is a mixture of a wild-type T7 DNA polymerase and a T7 DNA polymerase modified to have reduced 3′ to 5′ exonuclease activity.

84. The kit of claim 81, wherein said gene 4 protein is the 63-kDa form of the protein.

85. The kit of claim 84, further comprising a nucleoside diphosphokinase.

86. The kit of claim 85, wherein said nucleoside diphosphokinase is from *Escherichia coli*.

87. The kit of claim 79, further comprising inorganic pyrophosphatase.

88. The kit of claim 79, further comprises an ATP regeneration system.

89. The kit of claim 88, wherein said ATP regeneration system consists of phosphocreatine and creatine kinase.

90. The kit of claim 79, further comprising a T7 gene 6 exonuclease.

91. The kit of claim 79, further comprising a T7 DNA ligase.

92. The kit of claim 79, further comprising a single-stranded DNA binding protein of bacteriophage T7.

93. Method for overproducing the gene 4 protein of bacteriophage T7 comprising:

coproducing the gene 4 protein in *E. coli* with an agent that increases its level of production.

94. The method of claim 93, wherein said agent is a carboxy terminal fragment of gene 4 protein.

95. The method of claim 94, wherein said carboxy terminal fragment of gene 4 comprises the carboxy terminus of the protein after residue 221 (glutamine).

96. The method of claim 95, wherein said carboxy terminal fragment of gene 4 comprises the carboxy terminus of the protein between residues 260 (tryptophan) and residue 280 (serine).

97. Method of determining the amount of contaminating DNA in a sample comprising the steps of:

incubating said sample potentially containing contaminating DNA with a reaction mixture comprising a DNA polymerase such that if said contaminating DNA is present said DNA molecule is amplified to produce amplified product;

incubating at least one pre-determined amount of control DNA with said reaction mixture comprising a DNA polymerase such that said control DNA molecule is amplified to produce amplified control product; and

comparing the amount of amplified product in said sample with the amount of amplified control product as an indication of the amount of contaminating DNA in said sample.

98. The method of claim 97, wherein said DNA polymerase is from bacteriophage T7.

99. The method of claim 97, wherein said reaction mixture is at constant temperature.

100. The method of claim 97, wherein said reaction mixture further comprises a helicase, a primase and a single-stranded binding protein.

101. The method of claim 97, wherein said control DNA is amplified at least 10-fold.

102. The method of claim 97, wherein said reaction does not require exogenously-added oligonucleotide primers.

103. Method of claim 97, wherein amplification of said control DNA is exponential.

104. Method for amplifying DNA directly from cells, comprising the steps of:

lysing said cells to form a lysate; and

incubating said lysate in a reaction mixture comprising a DNA polymerase, a helicase, and a primase at a constant temperature, such that DNA present in said lysate is amplified to produce amplified product, wherein production of amplified DNA product does not require exogenously-added oligonucleotide primers.

105. The method of claim 104, wherein said cells are bacterial cells.

106. The method of claim 104, wherein said cells are human cells.

107. The method of claim 104, wherein said reaction mixture further comprises a single-stranded binding protein.

108. Method for amplifying DNA directly from cells comprising the steps of:

lysing said cells to form a lysate; and

incubating said lysate in a reaction mixture comprising a DNA polymerase such that DNA present in said lysate is amplified to produce amplified product, wherein production of amplified product does not require exogenously-added oligonucleotide primers, said amplification is exponential and wherein the amount of amplified product is at least ten–fold greater than the amount of DNA present in said lysate put into said reaction mixture.

109. The method of claim 108, wherein said cells are bacterial cells.

110. The method of claim 108, wherein said cells are human cells.

111. The method of claim 49, wherein said DNA is from bacteriophage.

112. A method of amplifying a plasmid template DNA molecule, comprising incubating said template DNA molecule in a reaction mixture comprising a DNA polymerase to produce amplified product, wherein production of amplified product does not require exogenously-added oligonucleotide primers, is exponential, and the amount of amplified product is at least 10-fold greater than the amount of template DNA put into the reaction mixture.

113. A method for preparing a DNA molecule for use in a DNA sequencing reaction comprising the steps of:

incubating said DNA molecule in a reaction mixture comprising a DNA polymerase to produce amplified product, wherein production of amplified product does not require exogenously-added oligonucleotide primers, is exponential,

the amount of amplified product is at least 10-fold greater than the amount of DNA molecule put into the reaction mixture; and

providing said amplified product for use in a DNA sequencing reaction.

114. The method of claim 108, wherein said amount of said amplified product

is at least 100-fold greater than the amount of DNA present in said lysate added to said reaction mixture.

115. The method of claim 108, wherein said amount of amplified product is at

least 1000-fold greater than the amount of DNA present in said lysate added to said reaction mixture.

116. The method of claim 112, wherein said amount of amplified product is at

least 100-fold greater than the amount of template DNA added to said reaction mixture.

117. The method of claim 112, wherein said amount of amplified product is at

least 1000-fold greater than the amount of template DNA present added to said reaction mixture..

118. The method of claim 113, wherein said amount of amplified product is at

least 100-fold greater than the amount of DNA added to said reaction mixture.

119. The method of claim 113, wherein said amount of amplified product is at

least 1000-fold greater than the amount of DNA added to said reaction mixture.

120. The method of claim 96, wherein said carboxy terminal fragment of gene 4 initiates at residue 271 (arginine).

121. A method for sequencing a nucleic acid sequence, wherein the DNA molecule to be sequenced is prepared in a constant temperature amplification reaction comprising the steps of:

incubating a single DNA molecule to be sequenced in a reaction mixture comprising a DNA polymerase to produce amplified product, wherein production of amplified product is done isothermally, is exponential, the amount of amplified product is at least 10-fold greater than the amount of DNA molecule put into the reaction mixture.

122. A method for sequencing wherein the DNA molecule to be sequenced is prepared in a reaction comprising the steps of:

incubating a single DNA molecule to be sequenced in a reaction mixture comprising a DNA polymerase to produce amplified product, wherein production of amplified product does not require exogenously-added oligonucleotide primers, is exponential, the amount of amplified product is at least 10-fold greater than the amount of DNA molecule put into the reaction mixture.

123. The method of claim 121, wherein said method of DNA preparing a DNA molecule for sequencing is performed isothermally.